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COMPARATIVE STUDIES ON THE MICROBIAL LOAD OF PLAIN AND FLAVOURED ICE CREAM

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SUMMARY

Eighty random samples of ice cream (20 each of plain, fruity, nutty and chocolate ice cream) collected from different localities in Giza and Cairo cities, were microbiologically examined to investigate and compare the effect of some natural added material on its microbial load. The obtained results in this study revealed that aerobic plate count were determined in different types of examined samples with a mean value ranged from $1.1x10^5 \pm 1x10^5$ to $5.8x10^5 \pm 1.6x10^5$, Enterobacteriaceae was detected in the different examined samples with a mean value ranged from $3.2x10^2 \pm 0.8\ x10^2$. Coliforms counts (MPN) in different types were present in percentage of 35, 60, 45 and 25% for plain, nutty, fruity and chocolate ice cream respectively. Staphylococci was present in different kinds of examined ice cream with mean values ranges from $8x102 \pm 0.48x10^2$ to $1.5x10^4 \pm 1.7x10^3$ while pseudomonas and Aeromonas were present in the examined samples with percentage ranges from 20 to 45%. Different species of Aeromonas could be identified in different percentage (*A.caviae, A.hinshawi, A.hydrophila*). Enteropathogenic Escherichia coli could be isolated from plain and fruity ice cream examined samples only.

Yersenia and Salmonella were failed to be detected in all examined samples of ice cream while Listeria species could be isolated in different percentage from the plain and fruity ice cream examined samples only.

The effect of addition of some types of natural materials on the microbial quality of ice cream, as well as public health importance of the detected microorganisms had been discussed.

INTRODUCTION

Ice-cream represents a congealed dairy product produced by freezing a pasteurized mixture of milk, cream, skimmed milk, sugars, emulsifiers and stabilizers. Other ingredients include flavouring matter as fruits, nuts, chocolate, candies and syrups are optionally added to enrich the flavour of ice-cream (Varnam and Sutherland, 1994).

Ice-cream, a milk-based product, is a good media for microbial growth due to high nutritive value, almost neutral pH value (6-7) and long storage duration (Bell and Kyriakides, 1998). However, pasteurization, freezing and hardering steps in the production of such product can eliminate most of the microbiological hazard, furthermore as automatic machines are commonly used for ice cream making in dairy industry the chance of contamination through direct hand manipulation can be reduced (Andreasen and Nielsen, 1998).

Due to the fact that most ice-cream is purchased by the consumer on basis of flavour and ingredients so there are several different flavours used in ice-cream manufacture which may be natural, artificial or fortified compound. Generally, this flavours are added to ice-cream by three ways during the manufacture process as in mix tank for liquid flavours and colours or variegating pump (for ribbons and ripples) or fruit feeder (for particulates fruit and nuts).

Nevertheless there are some steps in the production of ice cream can lead to the microbiological hazards. A major ingredients used to make this product are spoilage and several ingredients like flavours are added after the last lethal process, pasteurization, has been completed. Therefore, microorganisms are of considerable importance to the ice-cream industry (Marshall, 1998).

The rate of contamination and the hygienic quality of ice-cream are of great importance to the consumers therefore, this work was planned to evaluate the effect of different additives (flavours) on actual bacteriological hazard of ice-cream by comparing the microbiological load of plain and some types of flavoured ice-cream.

MATERIALS AND METHODS

1.Collection of Samples

Eighty random samples of ice-cream (20 each of plain, fruity, nutty and chocolate ice cream were collected from different dairy shops and supermarkets in its retail containers from Giza and Cairo cities. Collected samples were transferred to laboratory in an insulating icebox with a minimum of delay to be immediately examined for microbial load.

2. Preparation of Samples

Samples were prepared as the technique described by APHA (1992).

3. Enumeration of Different Types of Microorganisms

3.1. Aerobic Plate Count (APC) using the technique recommended by Swansonson et al (1992).

3.2.Enterobacteriacae Count: the technique adopted by Gork (1976) was applied using the drop plate method on Violet Red Bile Glucose Agar.

3.3.Staphylococci Count: Baird-Parker medium was used, and the suspected colonies were identified according to Balley and Scott (1982).

3.4.Pseudomonas and Aeromonas Count: using GSP medium and the technique described by Kielwein (1969). Suspected Aeromonas colonies were isolated, purified and identified according to Palumbo et al., (1992).

3.5.Coliform contents, the most probable Number (MPN) technique described by Htichine et al (1992) was used.

3.6. Enteropathogemc *E. Coli* counts using the recommended method of APHA (1992).

4. Isolation and identification of specific food borne pathogens

4.1. Salmonella species, the technique described by Flowers et al. (1992) was carried out.

4.2. Yersinia enterocolitica: The technique described by Donald and George (1992) was carried out.

4.3. Listeria monocytogenes: The technique recommended by APHA (1992) was followed. Suspected purified colonies of Listeria species showed positive Gram stain and catalase test were identified as described by Lovett (l987).

5. Data analysis

The obtained results were analyzed using computer program (Harp hard graphics and Excel office XP).

RSEULTS

Table (1): APC in different types of ice-cream (count cfu/ml)

Sample	No.of sample	Positive Samples		Min.	Max	Mean ± SE
		No.	%			
Plain ice-cream	20	20	100	$2.4X10^3$	$1X10^6$	$1.5X10^6 \pm 3.5X10^3$
Nutty ice-cream	20	20	100	$8.2X10^3$	$4.2X10^6$	$5.8X10^5 \pm 1.6X10^5$
Fruity ice-cream	20	20	100	$1X10^6$	$1X10^6$	$1.1X10^5 \pm 1X10^5$
Chocolate ice-cream	20	20	100	$4X10^2$	$1.2X10^5$	$2.6X10^4 \pm 0.8X10^5$

Table (2): Enterobacteriacea in different types of ice-cream (count cfu/ml)

Sample	No.of sample	Positive Samples		Min.	Max	Mean ± SE
		No.	%			
Plain ice-cream	20	9	45	$2X10^2$	$2.8X10^4$	$6X10^3 \pm 8.6X10^2$
Nutty ice-cream	20	13	65	$4X10^2$	$5.6X10^5$	$1.2X10^4 \pm 2.1X10^3$
Fruity ice-cream	20	11	55	10^2	$3.2X10^4$	$4X10^2 \pm 1.9X10^2$
Chocolate ice-cream	20	7	35	10^2	$2X10^3$	$3.2X10^2 \pm 0.8X10^2$

Table (3): Coliform content in different type of ice-cream (MPN)

Sample	No.of sample	Positive Samples		Min.	Max	Mean ± SE
		No.	%			
Plain ice-cream	20	7	35	70	$9X10^3$	$4X10^2 \pm 0.33X10^2$
Nutty ice-cream	20	12	60	$2.3X10^2$	$2.3X10^4$	$4X10^2 \pm 0.86X10^2$
Fruity ice-cream	20	9	45	40	$2.3X10^3$	$2.1X10^3 \pm 0.9X10^2$
Chocolate ice-cream	20	5	25	$2.3X10^2$	$9X10^3$	$6.5X10^2 \pm 2.6X10^2$

Table (4): Staphylococci count in different types of ice-cream (count cfu/ml)

Sample	No.of sample	Positive Samples		Min.	Max	Mean ± SE
		No.	%			
Plain ice-cream	20	11	55	$1.5X10^2$	$8X10^4$	$6X10^3 \pm 2.3X10^2$
Nutty ice-cream	20	12	60	$1.8X10^3$	$6.3X10^5$	$1.5X10^4 \pm 1.7X10^3$
Fruity ice-cream	20	8	40	10^2	$1.2X10^4$	$8X10^2 \pm 0.48X10^2$
Chocolate ice-cream	20	7	35	$2X10^2$	$5.2X10^3$	$4.6X10^3 \pm 1.9X10^2$

Table (5):Pseudomonas and Aeromonas count in different types of ice-cream (count cfu/ml)

Sample	No.of sample	Positive Samples		Min.	Max	Mean ± SE
		No.	%			
Plain ice-cream	20	8	40	$1X10^2$	$3X10^4$	$2.2X10^3 \pm 0.8X10^2$
Nutty ice-cream	20	9	45	$1.8X10^2$	$6.3X10^4$	$1.5X10^3 \pm 1.2X10^3$
Fruity ice-cream	20	5	25	10^2	$1.2X10^4$	$8X10^2 \pm 0.48X10^2$
Chocolate ice-cream	20	4	20	$2X10^3$	$6.3X10^4$	$4.5X10^3 \pm 1.9X10^2$

Table (6):Enteropathogenic Escherichia coli in different types of ice-cream (count cfu/fm)

Sample	No.of sample	Positive Samples		Min.	Max	Mean ± SE
		No.	%			
Plain ice-cream	20	2	10	$2.3X10^2$	$2.4X10^3$	$1X10^2 \pm 0.2X10$
Nutty ice-cream	20	0	0	<3	<3	<3
Fruity ice-cream	20	3	15	$1.5X10^2$	$2.1X10^3$	$1.3X10^2 \pm 0.4X10$
Chocolate ice-cream	20	0	0	<3	<3	<3

Table (7):Incidence of Listeria and Aeromonas species isolated from the examined ice-cream samples

		No.	%
Plain ice-cream	**Listeria species**	3	15
	L. welshimeri	2	10
	L. ivanovi	1	5
	Aeromonas species	4	20
	A. caviae	2	10
	A. hinshawii	1	5
	A. hydrophila	1	5
Nutty ice-cream	**Listena species**	0	0
	L. welshimeri	0	0
	L. ivanovi	0	0
	Aeromonas species	2	10
	A. caviae	1	5
	A. hinshawii	0	0
	A. hydrophila	1	5
Fruity ice-cream	**Listeria species**	4	20
	L. welshimeri	3	15
	L. ivanovi	1	5
	Aeromonas species	2	10
	A. caviae	1	5
	A. hinshawii	0	0
	A. hydrophila	1	5
Chocolate ice-cream	**Listena species**	0	0
	L. welshimeri	0	0
	L. ivanovi	0	0
	Aeromonas species	1	5
	A. caviae	1	5
	A. hinshawii	0	0
	A. hydrophila	0	0

DISCUSSION

Results recorded in table (1) revealed that the mean APC in different brands of examined ice-cream were $1.5x10^5 \pm 3.5x10^4$, $5.8x10^5 \pm 1.6x10^5$, $1.1x10^5 \pm 1x10^5$ and $2.6x10^4 \pm 0.8x10^5$ CFU/ml for plain ice-cream, nutty ice-cream, fruity ice-cream, and chocolate ice-cream respectively. From these obtained results, it was clear that chocolate as natural additives material give some degree of inhibition on survival of microorganisms in ice-cream. On the other hand, nuts and fruits as natural additives considered the most valuable source for contamination of such product with different types of microorganisms (Maifreni et al., 1993).

Tabulated results in table (2) explain that Enterobacteriaceae was detected in 7 samples of chocolate ice-cream with a percentage of 35%. The mean values of Enterobacteriaceae in the examined samples ranged from $3.2x10^2 \pm 0.8x10^2$ to $1.2x104^5 \pm 2.1x10^3$ CFU The low percentage of the Enterobacteriaceae in chocolate ice-cream if it compared with the other kinds of examined samples may be attributed to the inhibitory effect of chocolate during production of ice-cream (Kambamanoli-Dimou, 2000). Nearly similar results were recorded by Windrantz and Arais (2000) who confirm the inhibitory effect of chocolate on the growth of different types of microorganisms.

Inspection of the results in table (3) revealed that coliform content in different types of ice-cream (MPN) were present in a percentage of 35, 60, 45 and 25% with a mean values of $4x10^2 \pm 0.33x10^2$, $4x10^2 \pm 0.86$ $x10^2$, $2.1x10^3 \pm 0.9x10^2$ and $6.5x10^2 \pm 2.6x10^2$ CFU for plain ice-cream, nutty ice-cream, fruity ice-cream, and chocolate ice-cream respectively. The coliforms seems to be implicated in food illness. E. coli induces severe diarrhea in infants, cystitis, pyelonephritis as well as food poisoning among consumers (Sinell et al., 1989). Nuts additives was the most important source of contamination of ice-cream with coliforms (60%), on the other hand chocolate flavour considered as a good additive which has an inhibitory effect on microorganisms that may be present in ice-cream. These obtained results were in accordance with that reported by Wilson et al., (1997).

Results demonstrated in table (4) revealed that mean value of Staphylococci count in different kinds of ice-cream were $6x10^3 \pm 2.3x10^2$, $1.5x10^4 \pm 1.7x10^3$, $8x10^2 \pm 0.48x10^3$, and $4.6x10^3 \pm 1.9x10^2$ CFU with a percentage of 55, 60, 40 and 35% for plain, nutty, fruity, and chocolate ice-cream respectively. The obtained results were nearly similar to those reported by Bastepe and Kosker (1981).

Results in tables (5 & 7) deal with count of Pseudomonas and Aeromonas in different types of examined ice-cream samples as well as identification of the isolated Aeromonas species. From results in table (5) it was clear that the mean count of Pseudomonas and Aeromonas were ranged from $8x10^2 \pm 0.48x10^2$ to $4.5x10^3 \pm 1.8x10^3$ with a percentage from 20-45% in different types of examined samples. The chocolate ice-cream showed the lowest count rather than other types of ice-cream and this may be an indication for the inhibitory effect of chocolate as a natural additives if it compared with the other additives (nuts and fruits) (Murdough et al., 1996).

Results in table (7) demonstrate the incidence of Aeromonas species which could be isolated from the examined ice-cream samples. A. caviae could be isolated in percentage of 10, 5, 5 and 5% from plain, nutty, fruity, and chocolate ice-cream respectively. A. hinshawii could be isolated only from plain ice-cream in a percentage of 5%, while A. hydrophila could be isolated in percentages of 5% from plain, nutty and fruity each type of the examined samples, while it failed detection in chocolate ice-cream. Similar results were nearly recorded by Borneff and Kietzmann (1975).

Inspection of data in table (6) revealed that EPEC could be detected in plain and fruity ice-cream with a mean values of $1x10^2 \pm 0.2x10$ and $1.3x10^2 \pm 0.4x10$ with a percentage of 10 and 15% respectively.

Yersinia and Salmonella species failed to be detected in all examined samples of ice-cream, while Listeria species could be detected in different percentage from two types of examined ice-cream samples as it is obvious in table (7) in plain ice-cream Listeria species isolated in a percentage of 15% (10% for *L. welshimeri* and 5% for *L. ivanovi*), while in fruity ice-cream Listeria was isolated in a percentage of 20% (15% for *L. welshimeri* and 5% for *L. ivanovi*). Listeria organisms has the ability to repair the sub-lethal injury which may be caused by freezing of ice-cream and regain the capability to multiply when favourable growth conditions are available (Flanders and Donelly, 1994 and Hassan, 1996).

In conclusion, usage of selective natural additives may be a possible way for controlling microbial hazard of ice-cream by getting benefits from its inhibitory effect on some group of microorganisms. On the other hand, addition of some natural additives may lead to bad adverse results, leading to maximize the microbial load of ice-cream, consequently it is important from the health point of view to select the type, origin and characters of the material which may be added to ice-cream as well as application of good hygienic measures during production, storage and distribution of ice-cream is essential point to prevent the risk of human hazard.

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